



**Depth of Etch Comparison Between Self-limiting and
Traditional Etchant Systems**

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for the Degree of

MASTER OF SCIENCE

By

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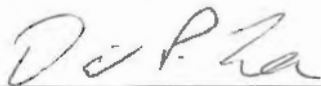
June 18, 2016

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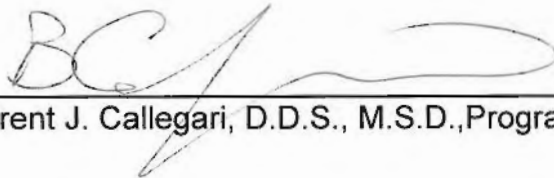
**Depth of Etch Comparison Between Self-limiting and
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Sara M. Wilson

APPROVED:



David P. Lee, D.M.D., M.S., Supervising Professor and Chairman

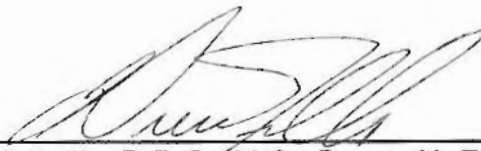


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A handwritten signature in black ink, appearing to read 'Sara M. Wilson', with a long, sweeping horizontal line extending to the right.

Sara M. Wilson
Tri-Service Orthodontic Residency Program
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Uniformed Services University
25 May 2016

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I would like to thank Dr. Wen Lien for his dedication to research and his assistance in piloting this study. Thank you to Dr. David Lee for his continued mentorship not only throughout my research endeavors but during my two years of residency as well.

DEDICATION

I would like to dedicate this thesis to my family.

ABSTRACT

Purpose: This study compared self-limiting phosphoric etch to traditional phosphoric etch to validate the self-limiting claim by measuring the depth of etch at multiple time intervals. **Methods:** Twenty-five bovine teeth were mounted and etched on the facial surface with the two different etchants (Ultradent's Opal Etch 35%, a self-limiting phosphoric acid, or 34% Tooth Conditioning Gel by Dentsply) at varied time intervals of 15, 30, 60, 90, 120 seconds. The teeth were scanned using Keyence 3D Laser Confocal Scanning Microscope prior to etching and scanned again after the etching to compare enamel height and calculate depth of etch. **Results:** A two-way ANOVA found that there was a significant difference between Opal versus Dentsply and there was also a significant difference between etch time. There is no significant difference between the interaction of etch material and etch time. **Conclusion:** The depth of etch of Opal etchant was consistently less than Dentsply etchant but continued to etch and therefore did not prove self-limiting.

TABLE OF CONTENTS

TITLE PAGE.....	i
ACKNOWLEDGEMENTS.....	iii
DEDICATION	iv
ABSTRACT	v
TABLE OF CONTENTS	vi
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
I. BACKGROUND.....	1
A. Introduction	1
II. OBJECTIVES	5
A. Purpose of Study	5
B. Hypothesis.....	5
C. Null Hypothesis.....	5
III. MATERIALS AND METHODS.....	6
A. Mounting the Teeth.....	7
B. Preparing the Teeth for Etching	7
C. Depth of etch scan.....	8
D. Measured Depth of Etch	9
E. Statistical Analysis	10
F. Figures of Materials and Methods Procedures.....	11
IV. RESULTS.....	20
V. DISCUSSION.....	27
VI. CONCLUSIONS.....	30
VII. LITERATURE CITED	31

LIST OF TABLES

<u>Table 4-1 Average depth of etch for Dentsply and Opal at each etch time</u>	22
<u>Table 4-2 Average maximum depth of etch for Dentsply and Opal at each etch time</u>	23
<u>Table 4-3 Significant difference for etch material and etch time for depth of etch</u>	24
<u>Table 4-4 Significant difference for maximum depth of etch for etch material and etch time</u>	24

LIST OF FIGURES

<u>Figure 1-1 Screenshot of Ultradent's website.....</u>	4
<u>Figure 3-1 Overview of Research Design.....</u>	7
<u>Figure 3-2 NSK Ultimate XL benchtop handpiece with NTI double-sided serrated diamond disk</u>	12
<u>Figure 3-3 Mounted bovine tooth facial surface exposed in ring and white orthodontic stone.....</u>	12
<u>Figure 3-4 Buehler Ecomet 3 used to polish the bovine teeth</u>	13
<u>Figure 3-5 Six ring samples being polished on Buehler Ecomet 3.</u>	13
<u>Figure 3-6 Polished samples with square enamel area bordered by tape to allow testing of both etch types on a single tooth to minimize variation of enamel rods.</u>	14
<u>Figure 3-7 Keyence 3D Laser Confocal Scanning microscope used for scanning samples</u>	14
<u>Figure 3-8 Sample lined up with tape marker on microscope.....</u>	15
<u>Figure 3-9 Dentsply 34% etch on left taped off area</u>	15
<u>Figure 3-10 Filtered Water</u>	16
<u>Figure 3-11 Water bottle to rinse etch off samples.....</u>	16
<u>Figure 3-12 Opal 35% phosphoric acid etch on right taped off area</u>	17
<u>Figure 3-13 Pre-scan.....</u>	17
<u>Figure 3-14 Post scan</u>	17
<u>Figure 3-15 Pre and post images overlaid.....</u>	18
<u>Figure 3-16 Sample #27, 15 s group. left side is Dentsply, right side is Opal..</u>	18
<u>Figure 3-17 Sample #22, 30 s group. left side is Dentsply, right side is Opal..</u>	18
<u>Figure 3-18 Sample #5, 60 s group. left side is Dentsply, right side is Opal....</u>	19
<u>Figure 3-19 Sample #8, 90 s group. left side is Dentsply, right side is Opal....</u>	19
<u>Figure 3-20 Sample #13, 120 s group. left side is Dentsply, right side is Opal</u>	19
<u>Figure 4-1 Mean Etching Depth</u>	25
<u>Figure 4-2 Mean of Maximum Etching Depth</u>	25
<u>Fig 4-3 SEM scan (5,000x magnification) 30 sec Dentsply on left, Opal on right</u>	26
<u>Fig 4-4 SEM scan (5,000x magnification) 120 sec Dentsply on left, Opal on right</u>	26

I. BACKGROUND

A. Introduction

Dental materials are constantly evolving to improve quality, efficiency and safety. Investigators such as Buonocore and Silverstone have led to improvements in dentistry to include resin and etchant. This thesis will focus on acid etch and its importance in dentistry. Without etchant, dental bonding would never have evolved to the point in which it is at now. This important material allows the Orthodontist to bond brackets to teeth and apply forces to move teeth without the brackets debonding from the teeth.

A break-through in dentistry occurred in 1955 when Buonocore reported that using 85% phosphoric acid etchant intraorally significantly increased duration of acrylic resin adhesion to enamel (Buonocore 1955). This study is the building block to lasting dental adhesion. Since that time a variety of etchants have been used along with different concentrations and recommended etchant duration. Some of these etchants include hydrofluoric acid, citric acid, hydrochloric acid, maleic acid, and nitric acid. Phosphoric acid has been demonstrated to be the most effective at promoting dental materials to adhere to enamel in vitro (Gwinnett 1971).

Historically, a series of different acid solutions were investigated for their effect on human enamel in vitro. The results showed that an unbuffered solution of 30% phosphoric acid produced the most favorable conditions (Silverstone, 1974).

The enamel surface changes in two distinct ways with acid etching. First, a shallow layer of enamel is removed by etching. In this manner, plaque, surface and sub-surface cuticles are effectively removed from the site to be bonded. In addition,

chemically inert crystallites in surface enamel are also removed, so favoring attempts at chemical union between hard tissue and resin. Second, the remaining enamel surface is rendered porous by the acid solution. It is into this porous region that the resin can penetrate and so bond with the enamel (Silverstone, 1974).

Acid etching removes approximately 10 microns of enamel surface and creates a morphologically porous layer (5 microns to 50 microns deep) (Lopes, 2007). In 1975, Silverstone reported three different types of etch patterns on enamel surfaces after acid etching. Type 1 has preferential dissolution of enamel cores, Type 2 had preferential dissolution of enamel prism peripheries, and Type 3 pattern could not be related to prism morphology and randomly occurred. It was determined that Type 1 and 2 patterns were preferred to retain adhesives on enamel surface by micromechanical interlocking. (Silverstone et al, 1975). The subsequent development of acid-etching technique was based on the Type 1 and/or Type 2 patterns by optimizing the types, concentration, and etching duration of the acid etchant.

Another parameter that has been investigated is the ideal amount of time to etch the tooth. Etching the normal intact enamel of adult teeth with 30%-50% phosphoric etch for 60 seconds was accepted as the protocol for enamel adhesion since the early 1980s (Zhu et al. 2014). It has since been shown that etching time of 15-20 seconds is equally effective (Gwinnett et al. 1992). Certain situations may dictate a variation in etchant times. For example, deciduous teeth require 120 seconds of acid etching to achieve the same etching pattern as adult dentition due to

deciduous enamel having lower mineral content and higher internal pore volume (Silverstone 1974, Angmar et al 1963).

It is clinically important not to over-etch the tooth. Over etching occurs beyond 60 seconds resulting in compromised tooth structure and bond strength (Wang et al. 1991). Confocal microscopy provides a way to measure depth of etch and ideal depth of etch is gauged to be 5-50 microns (Sturdevant, 2002).

There have been many studies that have measured depth of etch in either dentin or enamel. Some of the methods used have been scanning electron microscopy, contact profilometer, and the non-contact profilometer (Barkmeier 2009, Reis 2004, Legler 1990). The latest development in depth measurement is the confocal laser scanning microscopy. Confocal laser scanning microscopy combines the laser scan with a capture of a traditional visible light microscope image, producing a detailed 3D image of the surface. This has been shown to be a reliable method of measuring enamel erosion (Paepegaey, 2013).

In 2006, a new product, Ultradent Opal Etch, 35% phosphoric acid gel, was introduced and marketed as self-limiting. Opal etch is self-limiting in its depth of etch stating an average depth of etch 1.5 microns with 15 seconds etch in their instruction manual. The recommended directions are to apply to enamel for 30 seconds. Ultradent has made the following claims on their website:

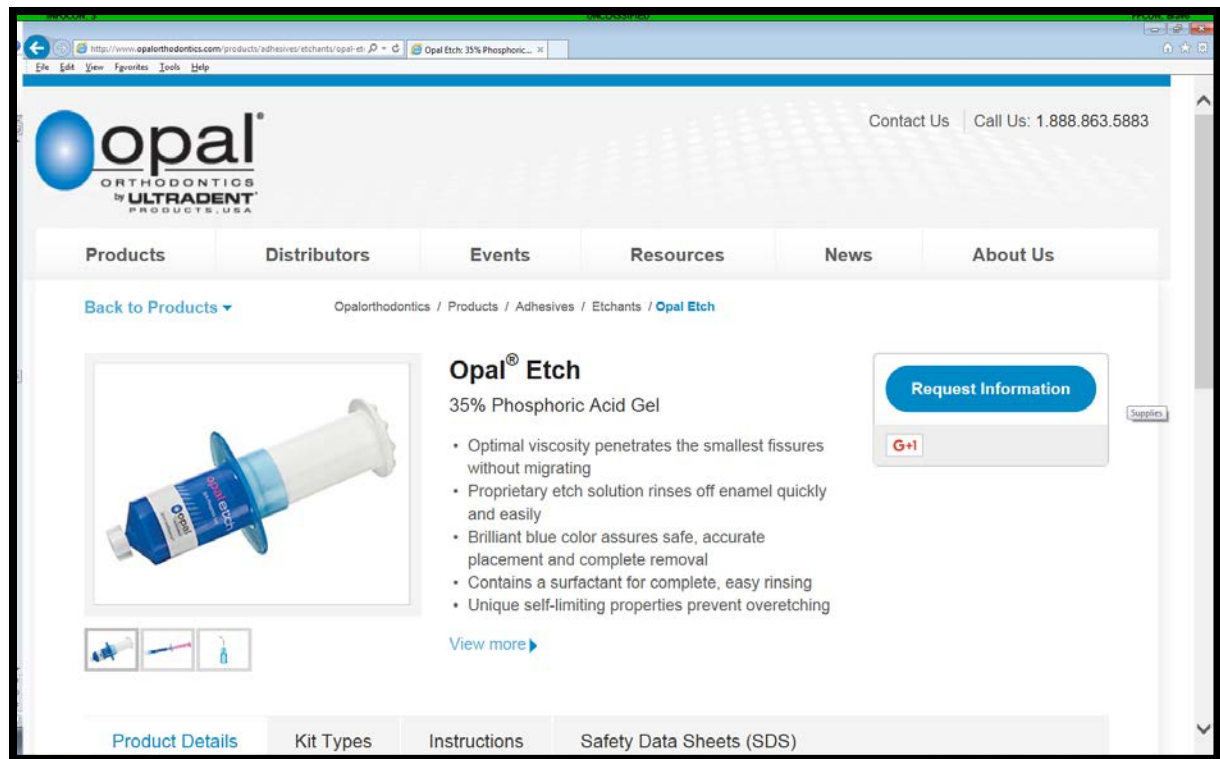
<http://www.opalorthodontics.com/products/adhesives/etchants/opal-etch>

- Optimal viscosity penetrates the smallest fissures without migrating
- Proprietary etch solution rinses off enamel quickly and easily
- Brilliant blue color assures safe, accurate placement and complete removal

- Contains a surfactant for complete, easy rinsing
- Unique self-limiting properties prevent over-etching

There is currently no publically available peer reviewed research supporting this self-limiting claim.

Figure 1-1 Screenshot of Ultradent's website



II. OBJECTIVES

A. Purpose of Study

To compare the self-limiting claim of Opal 35% phosphoric etch to a traditional Dentsply 34% phosphoric etch at 15, 30, 60, 90, 120 second time intervals by measuring the depth of etch on the facial surface of bovine teeth.

B. Hypothesis

There is a difference in depth of etch between self-limiting phosphoric acid etchant and traditional etchant as etching time increases.

C. Null Hypothesis

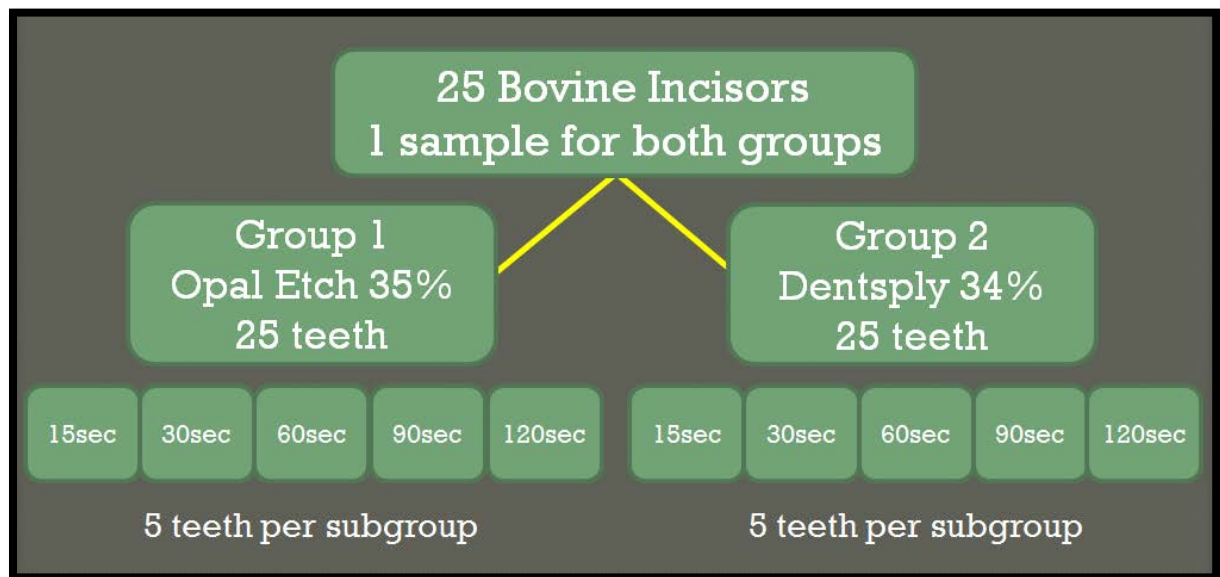
There is no difference in depth of etch between self-limiting phosphoric acid etchant and traditional etchant as etching time increases.

III. MATERIALS AND METHODS

One hundred bovine maxillary incisors were purchased from Animal Technologies, Inc, a USDA licensed slaughter facility, Tyler, Texas with the intent of using only 25 teeth. Once extracted, the frozen teeth were shipped in dry ice via FedEx. Upon arrival, the teeth were immediately stored and frozen for one week until they could be prepared for testing. Specimens with internal tooth defects, extensive craze lines, cracks, or chips were removed from the test sample. At the end of the week, the specimens were transferred to a 0.5% chloramine-T solution (using distilled water) and stored at room temperature (method recommended by Shade 2014). The 25 teeth were selected to be tested for depth of etch.

An organizational overview of the research project can be viewed in Figure 3-1. In order to prepare the bovine teeth, they were removed from the chloramine solution and sectioned by removing the root at the CEJ from the crown using a high speed handpiece (NTK, Ultimate XL) and diamond disk (NTI double-sided serrated diamond disk #D365-220) (Figure 3-2).

Figure 3-1 Overview of Research Design



A. Mounting the Teeth

Immediately after sectioning, each tooth was placed facial surface down on packing tape sticky side up to prevent dislodging. A 1.25" circular jig (Buehler) was then placed around the tooth and white dental stone (White Orthodontic Stone, Whipmix, Louisville, KY) was poured into the jig and allowed to set (Figure 3-3). Once set, the tape was removed and the mounted specimens were placed back into the 0.5% chloramine-T solution and stored at room temperature.

B. Preparing the Teeth for Etching

The mounted samples were polished using a Buehler Ecomet 3 (Buehler Ltd., Lake Bluff, Ill.) (Figure 3-4). Six jig samples at a time were polished with the following technique (250 RPM, water, 10 pounds pressure): 400 grit paper for 10 minutes, 600 grit for 10 minutes, 800 grit for 10 minutes, 1000 grit for 20 minutes (Buehler Carbimet silicon carbide paper 8" diameter) to achieve a smooth, even

surface for testing (Figure 3-5) . The resulting samples had top and bottom surfaces parallel with each other and a smooth facial surface accompanied with a smear layer. After polishing enamel, it was verified that enamel was still present and that dentin was not exposed by visual inspection without magnification. The two etchant groups of 25 teeth, were subdivided into 5 subgroups of 5 specimens. To minimize variation between tooth samples, the same tooth was used for both the Dentsply and Opal etchant samples. The polished facial surface was divided into two square samples roughly 2mm x 2mm using blue adhesive tape (3M Company, St. Paul, Minn) (Figure 3-6).

C. Depth of etch scan

The samples were scanned using 3D Laser Confocal Scanning Microscope (VK-X210/X200K, Keyence, Itasca, IL, USA) prior to etching to provide a baseline to compare the post etch scan (Figure 3-7). To ensure repeatability of specimen's positioning, tape was applied to the ring and to the base of microscope with marked lines. These lines allowed for a repeatable position between the pre and post scan. (Figure 3-8). The scanning parameters included an initial 10x magnification to capture the entire polished surface for ease of identifying landmarks followed by increasing to 20x magnification. The software commands were set to: specify the area (height range>manual, laser intensity=auto), for noise reduction. Each corner of the image was set to confocal upper and lower limits, followed by saving the initial image.

Once the initial scan was complete, the sample was etched in the first square (left side) with Dentsply for the designated amount of time (Figure 3-9), then rinsed

with a water bottle filled with Milli Q filtered water for ten seconds (Figures 3-10, 3-11), and blotted dry with a Kimtech chem wipe (Kimwipes science wipes, Kimberly Clark, Dallas, TX). Immediately followed by etching the neighboring right square of enamel with Opal etch for the same amount of time and followed by the same rinsing and drying regiment (Figure 3-12). Immediately after etching both squares, a post scan was completed in exactly the same manner as the initial scan.

Each subgroup consisting of five samples was etched with phosphoric acid using both Dentsply and Opal Etchant for the subgroup's designated time (15, 30, 60, 90, and 120 seconds). Each tooth followed the procedure described above from beginning to end with the only variations being etchant time and etchant type as established by this study.

D. Measured Depth of Etch

Image processing was performed prior to measuring depth of etch. Measurements were recorded at six different lines throughout the etched area for each Dentsply and Opal etch. The area at each line was calculated and the average of all measured points was taken to determine the depth of etch to the nearest micron. Results were collected after matching the pre-scan to the post-scan with the Multi File software. Filters/reference planes were performed on every image to include selection of the following predetermined options from the program: Smoothing Gaussian, size 7x7, height cut level strong, DCL=10 (DCL = dark cut level = this eliminates areas where there is no laser reflected such as a hole), BCL=65500 (BCL = bright cut level = this eliminates areas where reflected laser intensity is too great such as a saturated image). For example: Smoothing Gaussian parameter

reduced noise from images by using a Gaussian filter in a 7x7 pixel size and then height cut level provided further noise reduction which eliminated signals above 65500 and below 10. The images were then unified using batch height range settings and selecting the pre and post scan together. The scans were matched by selecting a few distinct irregularities on the un-etched portion of the enamel and matching the two scans on them in the x, y, and z axis (Figures 3-13 – 3-15). Then a horizontal line was dropped through the sample and an area was selected for both etch types which measured depth. Two different measurements were obtained: surface area depth (width x depth) and maximum depth (single deepest point). The width of these etched areas was recorded and provided a depth measurement extracted from the surface area. In addition to this measurement a maximum depth was extracted which provided the single deepest measurement. The horizontal line was dropped at six different points which allowed an average of the etched surface to be calculated for the entire sample instead of a single point. This measurement was calculated in microns. (Figures 3-16 – 3-20)

E. Statistical Analysis

Prior to beginning the research project, a power analysis was calculated and used to anticipate the likelihood that the research project will yield a significant effect, thus providing guidelines to accept or reject the null hypothesis. The power analysis determined that five samples per test group provided sufficient strength in this study. The larger the effect size used in the power analysis, the larger the sample size. Additionally, the more liberal the criteria needed for an alpha level, the higher the expectation is that the study will yield a statistically significant result.

A two-way ANOVA and Tukey's Post Hoc Test were selected to evaluate the two independent variables of etchant type (2-levels) and time (5-levels) ($\alpha = 0.05$). The only dependent variable is Depth of Etch measured in Microns (μ). The test evaluates the standard deviation (SD) among sample means, and then makes inferences about the differences between population means.

A one-way ANOVA and Tukey's Post Hoc Test was selected to determine if there was a significant difference based on time of etching between the two etchants. A Bonferroni correction was applied because of multiple comparisons between time groups and etchant material ($\alpha=0.025$).

A sample size of five teeth per group will provide 80% power to detect an Effect Size of 0.23 (approximately 0.46 standard deviation difference) among means for the main factor of etch material, and a small effect size of 0.29 (or approximately a 0.58 standard deviation difference) among means for the main factor of time and for the interaction term, when testing with a two-factor ANOVA at the alpha level of 0.05 (NCSS PASS 2002). After 3D Laser Confocal Scanning Microscope scanning results were recorded, all teeth were disposed of as bio-hazardous waste in accordance with occupational Safety and Health Administration (OSHA) regulations and standard military protocol. Standard safety precautions were followed while handling all teeth.

F. Figures of Materials and Methods Procedures

The images of the research procedures are listed and documented in order of their occurrence.

Figure 3-2 NSK Ultimate XL benchtop handpiece with NTI double-sided serrated diamond disk



Figure 3-3 Mounted bovine tooth facial surface exposed in ring and white orthodontic stone



Figure 3-4 Buehler Ecomet 3 used to polish the bovine teeth



Figure 3-5 Six ring samples being polished on Buehler Ecomet 3.

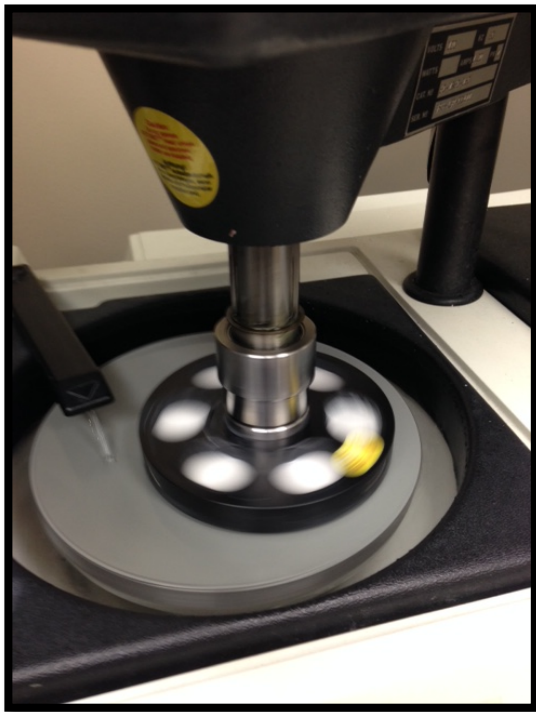


Figure 3-6 Polished samples with square enamel area bordered by tape to allow testing of both etch types on a single tooth to minimize variation of enamel rods.

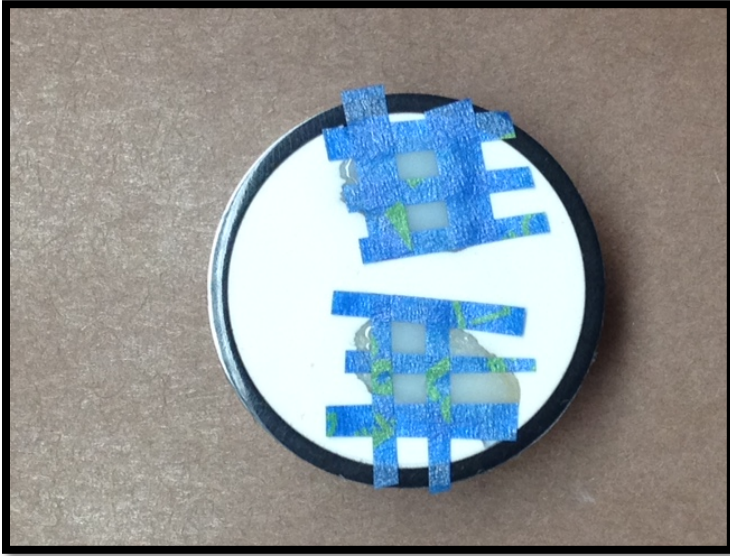


Figure 3-7 Keyence 3D Laser Confocal Scanning microscope used for scanning samples



Figure 3-8 Sample lined up with tape marker on microscope

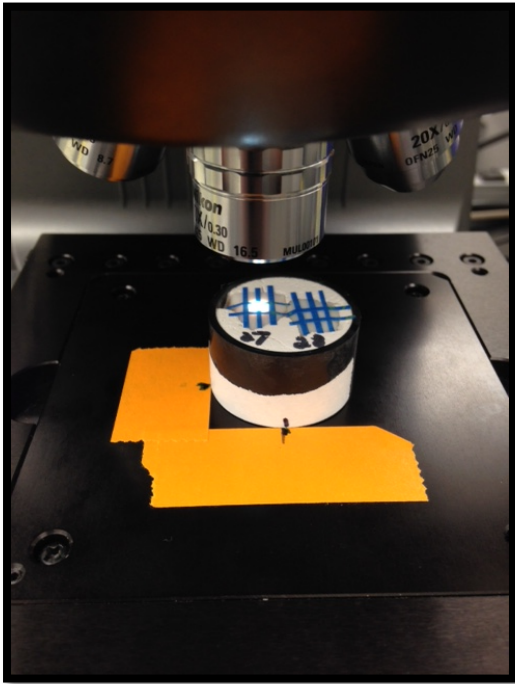


Figure 3-9 Dentsply 34% etch on left taped off area

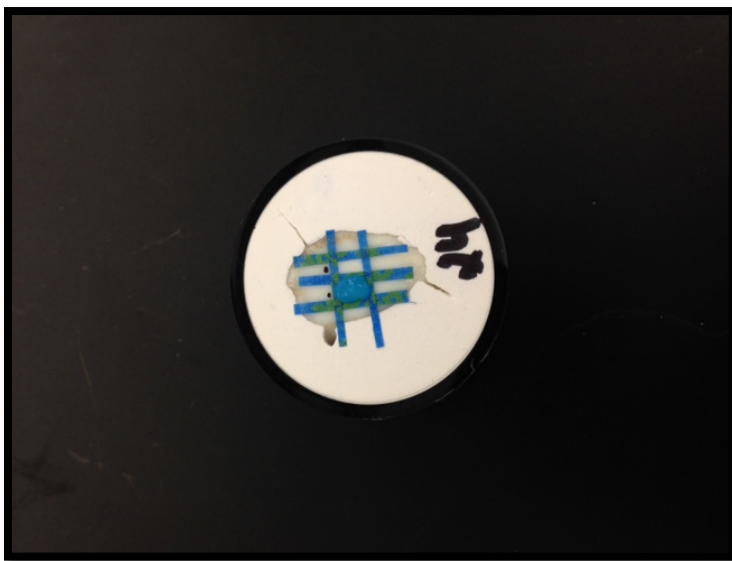


Figure 3-10 Filtered Water



Figure 3-11 Water bottle to rinse etch off samples



Figure 3-12 Opal 35% phosphoric acid etch on right taped off area

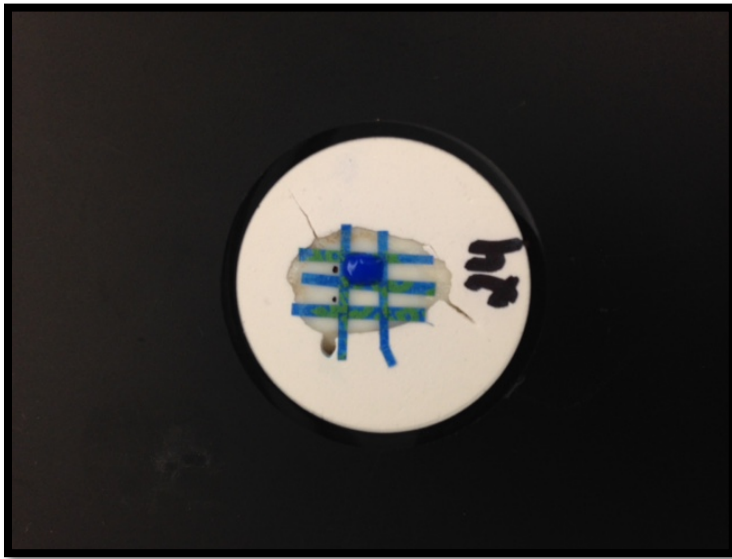


Figure 3-13 Pre-scan

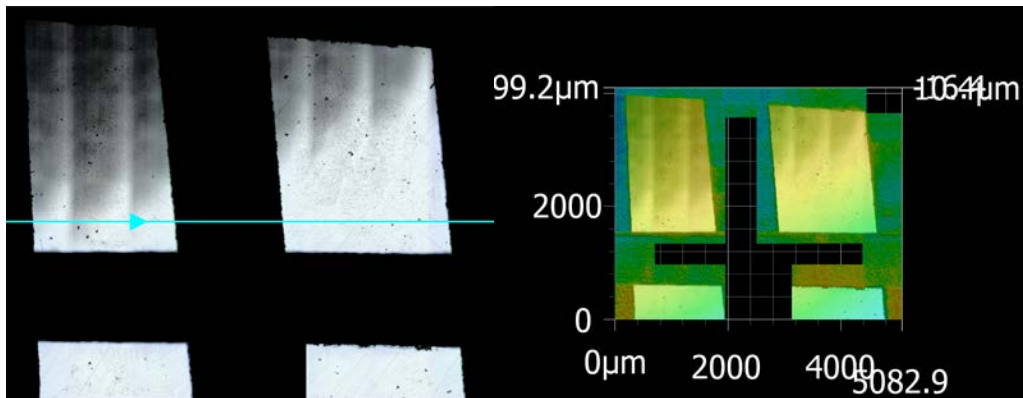


Figure 3-14 Post scan

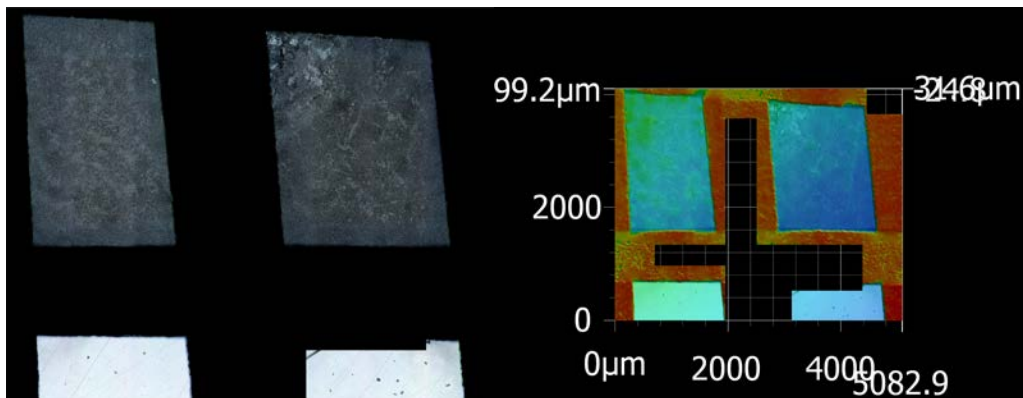


Figure 3-15 Pre and post images overlaid

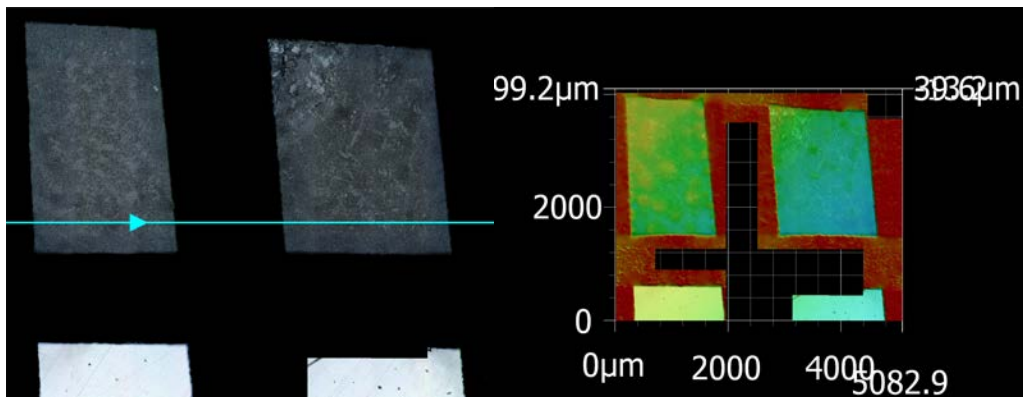


Figure 3-16 Sample #27, 15 s group. Left side is Dentsply, right side is Opal

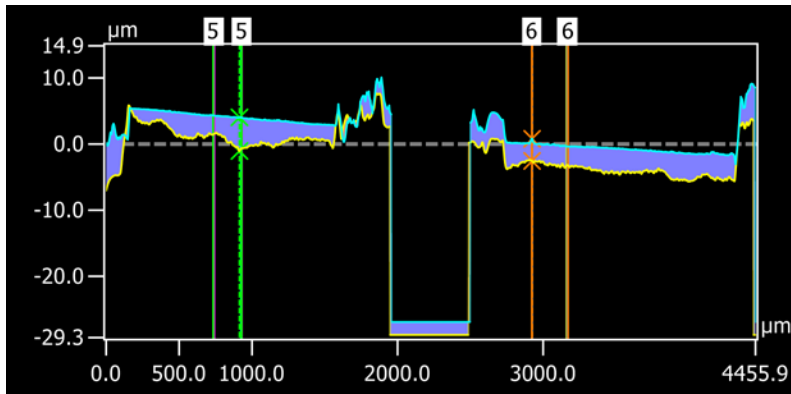


Figure 3-17 Sample #22, 30 s group. Left side is Dentsply, right side is Opal

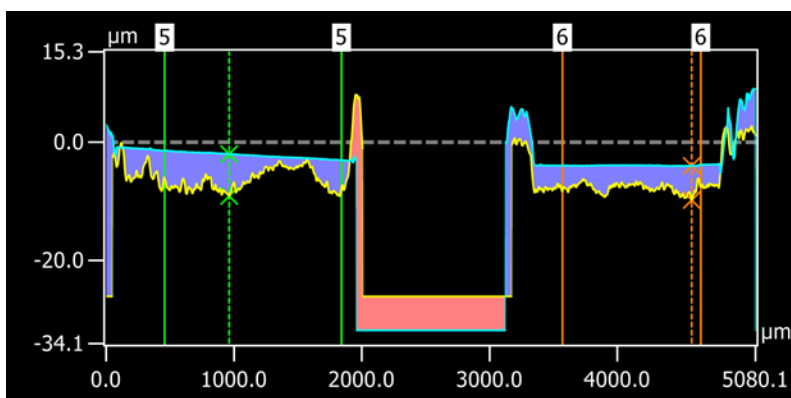


Figure 3-18 Sample #5, 60 s group. Left side is Dentsply, right side is Opal

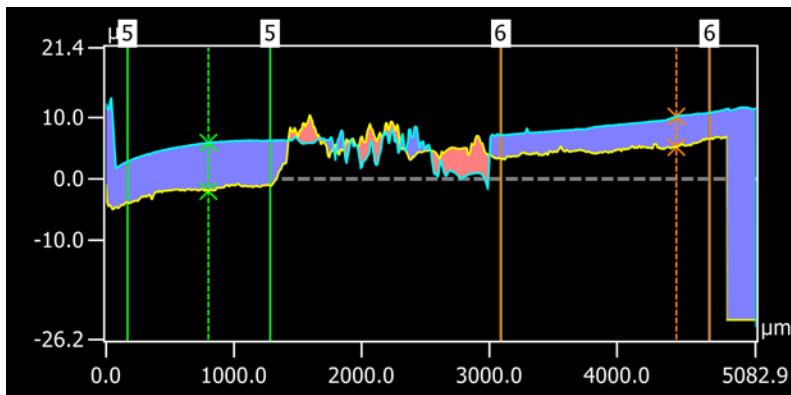


Figure 3-19 Sample #8, 90 s group. Left side is Dentsply, right side is Opal

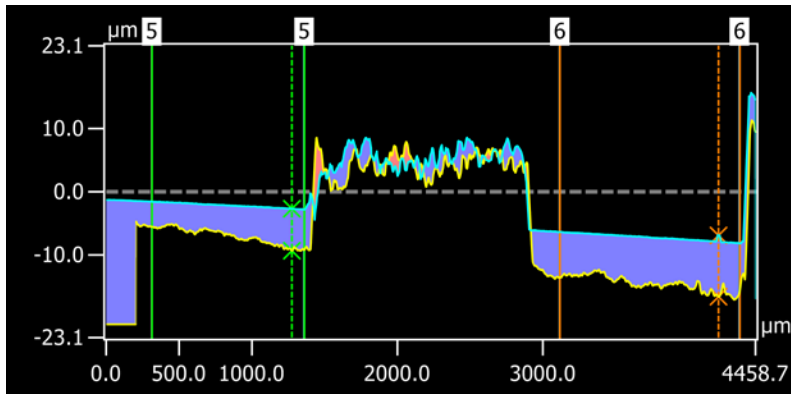
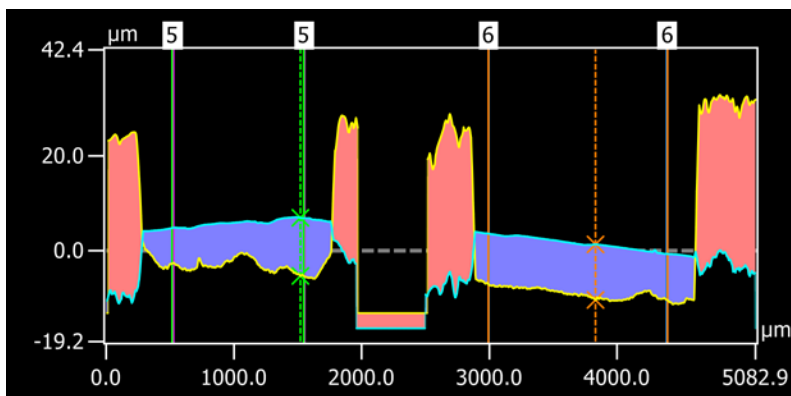


Figure 3-20 Sample #13, 120 s group. Left side is Dentsply, right side is Opal



IV. RESULTS

The mean etching depth and mean maximum etching extrapolated from six measurements for all samples is recorded in the following tables along with the average etching depth and average maximum etching and the standard deviation for each group and subgroup (Table 4-1 and Table 4-2). Opal consistently has less depth of etch than Dentsply at the corresponding etch times however this difference is not statistically significant.

Depth of etch increased progressively as etch time increased for both Opal and Dentsply (Figure 4-1 and 4-2). Due to this continued increase of depth of etch the self-limiting claim Opal makes for this etch is unfounded. It is no more self-limiting than traditional Dentsply etch.

Scanning Electron Microscope (SEM) images were obtained to allow for visual appreciation of the surface change with the different etch materials at different etch times (Figure 4-3 and 4-4). One sample was used (to minimize variations) to compare the 30 second etch time of both Dentsply and Opal versus the 120 second etch time of both Dentsply and Opal. These etch times were selected for comparison based on the statistically significance difference found during the Confocal Laser Microscopy depth measurement. The 30 second scan shows the enamel surface with the prism centers removed preferentially characterized as a honeycomb appearance or Type 1 etch pattern. The 120 second scan shows the enamel surface with the preferential removal of the prism peripheries termed a Type 2 etch pattern. Both etch types had similar appearance at the corresponding etch times.

The results of the two-way ANOVA found that the etch material (Dentsply and Opal) had overall significant differences and the overall etch time had significant differences. The interaction between etch material and etch time shows no significant difference. (Table 4-3 and 4-4). When comparing the different materials, Dentsply showed statistically significant greater etching depth when compared to Opal at etching time of 15 seconds ($p < 0.001$). Results of the Tukey's post hoc test shows that irrespective of material, etching time of 15 seconds or 30 seconds will have the same depth of etch or the etching time at 60, 90 or 120 seconds have the same depth of etch. However, etching time of 15 and 30 seconds are statistically significantly different than etching times 60, 90, and 120 seconds.

Table 4-1 Average Depth of Etch for Dentsply and Opal at each etch time

Descriptive Statistics

Dependent Variable: Depth of Etch

Etchmaterial	Etchtime	Mean (μm)	Std. Deviation	N
Dentsply	15 s	-3.99	.45	5
	30 s	-3.77	1.05	5
	60 s	-5.46	.54	5
	90 s	-5.73	.35	5
	120 s	-6.41	.55	5
Opal	15 s	-2.49	.50	5
	30 s	-2.77	.71	5
	60 s	-4.46	.78	5
	90 s	-5.01	.45	5
	120 s	-5.45	.78	5
Total	15 s	-3.24	.91	10
	30 s	-3.27	.99	10
	60 s	-4.96	.82	10
	90 s	-5.37	.53	10
	120 s	-5.93	.81	10

Table 4-2 Average Maximum depth of etch for Dentsply and Opal at each etch time

Descriptive Statistics

Dependent Variable:Max depth

Etchmaterial	Etchtime	Mean (μm)	Std. Deviation	N
Dentsply	15 s	-5.60	1.13	5
	30 s	-5.11	1.17	5
	60 s	-7.23	.54	5
	90 s	-7.50	.74	5
	120 s	-8.32	1.11	5
Opal	15 s	-3.74	1.23	5
	30 s	-3.64	.95	5
	60 s	-5.84	.71	5
	90 s	-6.50	.78	5
	120 s	-6.81	1.42	5
Total	15 s	-4.67	1.48	10
	30 s	-4.37	1.26	10
	60 s	-6.54	.94	10
	90 s	-7.01	.89	10
	120 s	-7.56	1.44	10

Table 4-3 Significant difference for etch material and etch time for depth of etch

Tests of Between-Subjects Effects					
Dependent Variable: Depth of Etch					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	75.218 ^a	9	8.358	19.689	.000
Intercept	1038.596	1	1038.596	2446.786	.000
Etchmaterial	13.387	1	13.387	31.538	.000
Etchtime	61.019	4	15.255	35.938	.000
Etchmaterial * Etchtime	.812	4	.203	.478	.752
Error	16.979	40	.424		
Total	1130.792	50			
Corrected Total	92.197	49			

a. R Squared = .816 (Adjusted R Squared = .774)

Table 4-4 Significant difference for maximum depth of etch for etch material and etch time

Tests of Between-Subjects Effects					
Dependent Variable: Max depth					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	108.313 ^a	9	12.035	11.659	.000
Intercept	1819.734	1	1819.734	1762.868	.000
Etchmaterial	26.112	1	26.112	25.296	.000
Etchtime	81.241	4	20.310	19.675	.000
Etchmaterial * Etchtime	.960	4	.240	.233	.918
Error	41.290	40	1.032		
Total	1969.337	50			
Corrected Total	149.603	49			

a. R Squared = .724 (Adjusted R Squared = .662)

Fig 4-1 Mean Etching Depth

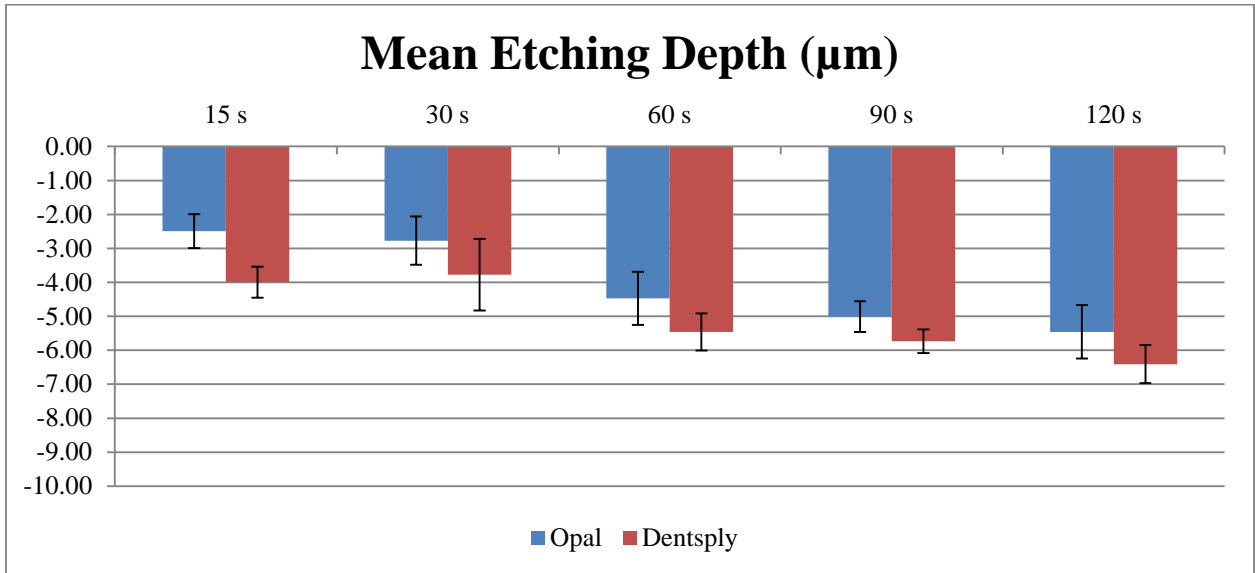


Fig. 4-2 Mean of Maximum Etching Depth

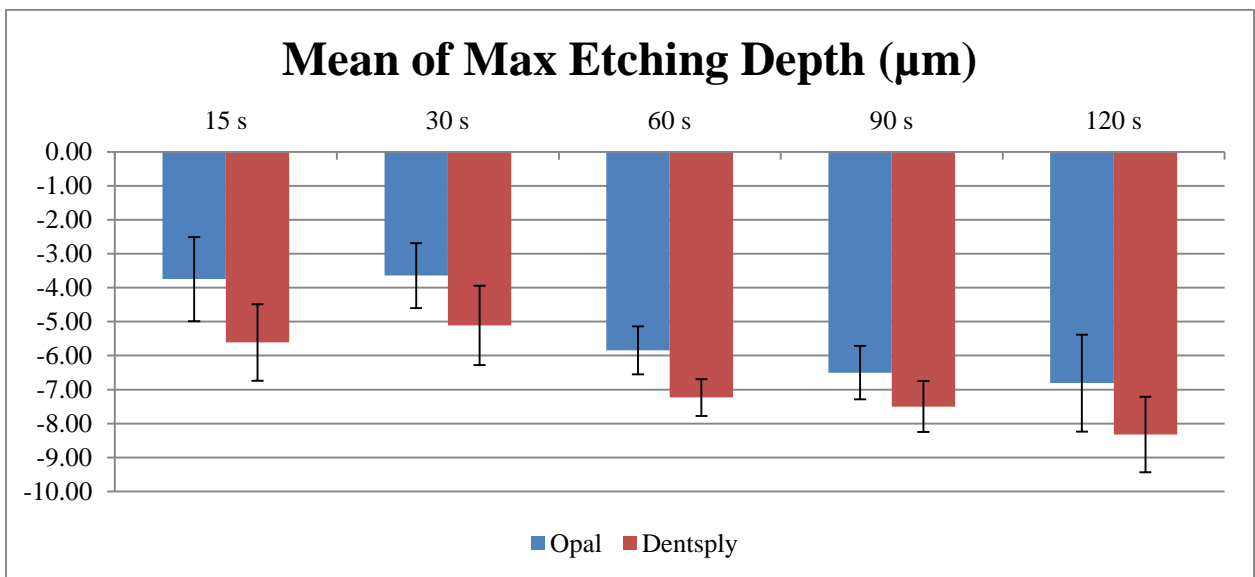


Fig 4-3 SEM scan showing enamel etching pattern type I after application of 30 sec etch: Dentsply on left, Opal on right. (5,000x magnification)

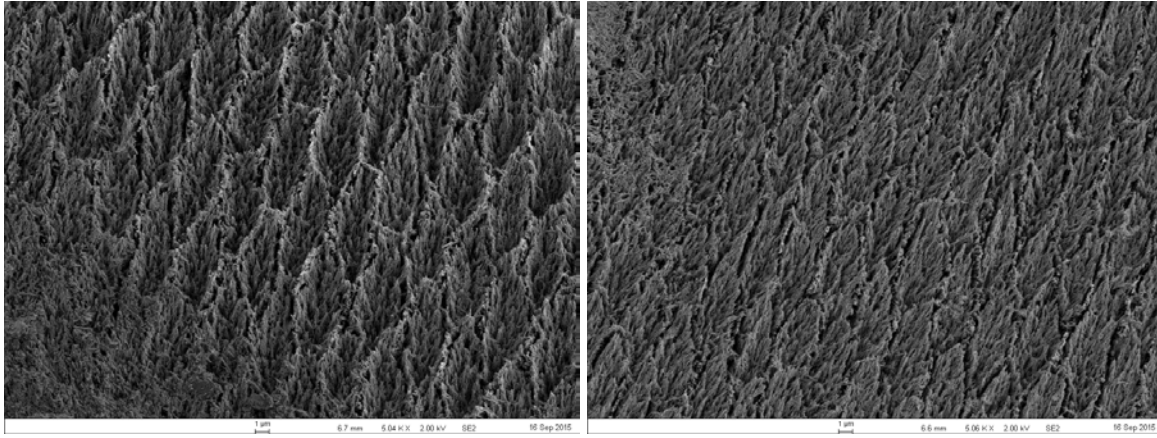
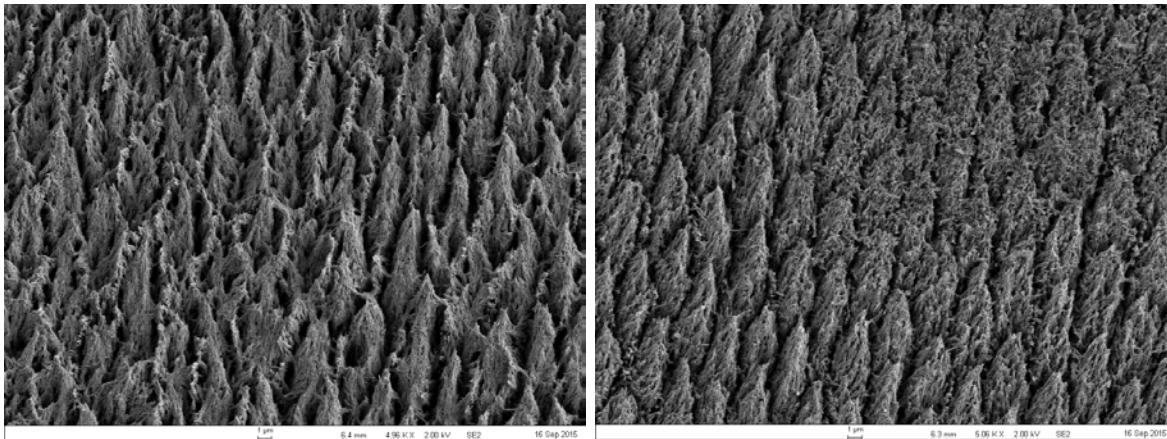


Fig 4-4 SEM scan showing enamel etching pattern type II after application of 120 sec etch: Dentsply on left, Opal on right. (5,000x magnification)



V. DISCUSSION

Over etching the tooth surface is of great concern in dentistry due to increased bond failures (Wang et al. 1991). It has been demonstrated that etching for more than 60 seconds, termed over-etching, significantly decreased bond strength and resulted in excess enamel being removed in the process (Barkmeier et al. 1986). The idea of having a self-limiting etch would be clinically beneficial to the Orthodontist for several reasons including the ability to etch an entire arch at one time without the risk of bond failures from over etching. In theory all dental etch is self-limiting since it does stop penetrating at some point due to the buffering effect that occurs with demineralization. However, over-etching still occurs resulting in collapsed enamel rods and therefore bond failures.

The etching of tooth enamel by phosphoric acid is itself somewhat self-limiting due to the high calcium content in enamel. Unless the etchant is constantly agitated, the calcium that is freed from the enamel hydroxyapatite surface while etching serves as a buffer (Driessens 1990). The viscous acid gel which is held together mostly by glycerin, sits on top of the enamel and the etching process is primarily a surface phenomenon. Demineralized surface calcium functions as a buffer to resist changes in hydrogen ion concentration. There is a gradient in the solubility of tooth enamel with depth. Excessive etching times of 120 seconds, therefore, changes the pattern of the enamel surface etch to a point where resin infiltration and entanglement is not as profound as the etch patterns at 30 to 90 seconds. If the enamel surface is being etched with a viscous gel and is not disturbed, the etching depth is limited by this buffering action (Driessens 1982).

Due to variations in surface chemistry and enamel texture, it is hard to compare teeth from different subjects. One tooth can be more calcified and when the surface is prepped will result in a thicker smear layer which will inadvertently result in less enamel being removed when etched. There are several unknowns regarding the samples used in this study such as age, diet, and environment of the subjects therefore the resulting in differing orientation of enamel prisms with differing reaction to acid conditioning (Lopes 2007). For this reason, one sample tooth was used to test both etch materials to minimize the variation in the enamel rod orientation.

There is only one company (Ultradent) that manufactures a self-limiting phosphoric acid etch to the authors knowledge at the time of this study. Ultradent produces two 35% phosphoric acid etchants, Ultra-etch and Opal Etch. According to company representatives, the etchants are exactly the same product simply marketed differently. Opal Etch being marketed to Orthodontists and Ultra-Etch being marketed to general dentists.

This study was geared towards comparing etchants of similar strength. Dentsply 34% phosphoric acid was selected as the control because it was similar in strength to Opal Etch 35% phosphoric acid. Both etchants were also tested to compare their acidity. Both etchants were similar in acidity with Opal Etch at pH 0.32 and Dentsply at pH 0.12. Every effort was made to ensure all parameters of the study were constant to evaluate the interaction between the two independent variables of etchant type (2-levels) and time (5-levels).

Confocal laser scanning microscopy (CLSM) was used for measuring enamel loss from acid etching via surface profile. It has been shown that CLSM is as reliable as other methods (contact profilometer and non-contact profilometer) for use in erosion measurement (Paepegaey et al. 2013). The reason why CLSM was selected for this study was due to the fact that it does not necessarily require fine sectioning and eliminates the cumbersome procedures of scanning electron microscopy. Moreover extremely high-quality images and three dimensional reconstructions can be obtained.

This study showed as etching time increased, depth of etch increased regardless of material. Opal Etch followed the same depth of etch progression as Dentsply with the corresponding etch time. The overall etch of depth for Opal was consistently less than Dentsply at the same etch time however this finding was not significant. It was also determined that etching at either 15 seconds or 30 seconds results in the similar depth of etch whereas etching at either 60, 90, or 120 seconds results in the similar depth of etch.

The results of the two-way ANOVA with a Tukey's post hoc found that the depth of etch of each material (Dentsply versus Opal) as well as etch time (15, 30, 60, 90, and 120 seconds) has statistically significant differences. There is no statistically significant difference between the interaction of etch material and etch time. Based on this data, the null hypothesis was accepted and it does not support the claim that Opal Etch is self-limiting.

VI. CONCLUSIONS

1. The null hypothesis was accepted in that Opal Etch is not self-limiting and will continue to etch the tooth if left on the enamel surface past 60 seconds.
2. Opal etchant generally had a lesser depth of etch than Dentsply but had a similar progression of depth as time increased.
3. Etch material had overall statistically significant difference. When all the data was collected for Dentsply regardless of time it was statistically different than all the data collected for Opal resulting in an overall difference between the etch materials.
4. Etch time had overall statistically significant difference. Regardless of etch material, different times result in different etch depth.
5. There is no statistically significant interaction between etch material and etch time. Etching enamel with Dentsply 34% phosphoric acid for 15 seconds is not significantly different than etching enamel with Opal Etch 35% phosphoric acid for 15 seconds.
6. Fifteen and thirty seconds had the similar depth of etch as did sixty, ninety, and one hundred and twenty seconds when comparing the same etch type.
7. The recommendation to clinicians is to continue following literature guidelines. Recommended etch time for 30-40% Phosphoric acid etch is 15-30 seconds. Adjust the etchant time as needed for individual teeth.

VII. LITERATURE CITED

1. Angmar B, Carlstrom D, Glas JE. Studies of the ultrastructure of dental enamel.
IV. The mineralization of normal human enamel. Journal of Ultrastructure
Research;1963:12-23
2. Aida M, Odaki M, Fujita K, Kitagawa T, Teshima I, Suzuki K, Nishiyama N.
Degradation-stage Effect of Self-etching Primer on dentin Bond durability. J Dent
Res; 2009:443-448
3. Barkmeier W, Erickson R, Kimmes N, Latta M, Wilwerding T. Effect of enamel
etching time on roughness and bond strength. Oper Den; 2009: 217-222
4. Barkmeier WW, Shaffer SE, Gwinnet AJ. Effects of 15 vs. 60 second enamel
acid conditioning on adhesion and morphology. Oper Dent 1986;11:111-6.
5. Brannstrom and Nordenvall, The effect of acid Etching on Enamel, Dentin, and
the Inner Surface of the Resin Restoration: A Scanning Electron Microscopic
Investigation. J Dent Res 56(8): 917-923 August 1977
6. Buonocore, MG. A Simple Method of Increasing the Adhesion of Acrylic Filling
Materials to Enamel Surfaces. J Dent Res;1955:849-853
7. Cartensen. Clinical effects of reduction of acid concentration on direct brackets.
Angle Orthod;1993:221-224.
8. Cehreli SB, Eminkahyagil N. Effect of Active Pretreatment of Self-etching Primers
on the Ultramorphology of Intact Primary and Permanent Tooth Enamel. Journal
of Dentistry for Children;2006:73-74

9. Dominguez T, Vicente de Moura L, Campanatti C, Catharino M. A comparative clinical study of the failure rate of orthodontic brackets bonded with two adhesive systems: Conventional and Self-Etching Primer (SEP). *Dental Press J Orthod*; 2013:55-60
10. Driessens FCM, Verbeeck RMH. *Biomaterials*. Boca Raton: CRC Press; 1990.
11. Driessens FCM. Mineral aspects of dentistry. In: Myers HM, editor. *Monographs in oral science*, Vol. 10. , Karger, Basel; 1982. p.139.
12. Güvenç B, Törün Ö, Devecioğlu J. Comparison of a recently developed nanofiller self-etching primer adhesive with other self-etching primers and conventional acid etching. *European Journal of Orthodontics* 31;2009:271–275.
13. Gwinnett AJ. Histologic changes in human enamel following treatment with acidic adhesive conditioning agents. *Arch Oral Biology*;1971:731-738
14. House and A. J. Ireland. An Investigation into the Use of a Single Component Self-etching Primer Adhesive System for Orthodontic Bonding: a randomized controlled clinical trial. *Journal of Orthodontics*, Vol. 33, 2006, 38-44
15. Hobson RS, Rugg-Gunn AJ, Booth TA. Acid-etch patterns on the buccal surface of human permanent teeth. *Archives of Oral Biology* 47;2002:407–412
16. Holzmeier M, Schaubmayr M, Dasch W, Hirschfelder U. A New Generation of Self-etching Adhesives: Comparison with Traditional Acid Etch Technique. *J Orofac Orthop*; 2008:78–93

17. Kinch, et al. A clinical trial comparing the failure rates of directly bonded brackets using etch times of 15 or 60 seconds. Am J Orthod Dentofac Orthop;1988:476-83.
18. Legler LR, Retief DH, Bradley EL. Effects of phosphoric acid concentration and etch duration on enamel depth of etch: An in vitro study. Am J Orthod Dentofacial Orthop 1990;140:154-160
19. Lopes GC, Thys DG, Klaus P, Oliviera GMS, Widmer N. Enamel Acid Etching: A Review. Compendium; 2007: 18-25
20. Lopes GC, Vieira LC, Araújo E, Bruggmann T, Zucco J, Oliveira G.
21. Effect of Dentin Age and Acid Etching Time on Dentin Bonding. J Adhes Dent 13; 2011:139-145
22. Paepegaey A, Barker M, Bartlett D. Measuring enamel erosion: A comparative study of contact profilometry, non-contact profilometry and confocal laser scanning microscopy. Dent Mat 29; 2013: 1265-1272
23. Perdigao, L. The effect of etching time on dentin demineralization. Quintessence Int; 2001:19-26.
24. Perdigao J. An Ultra-Morphological Study of Human Dentine Exposed to Adhesive Systems (thesis). Leuven, Belgium: Catholic University of Leuven;1995
25. Perdigao J, Lambrechts P, Meerbeek BV, Tome AR, Vanherle G, Lopes AB. Morphological field emission-SEM study of the Effect of Six Phosphoric Acid Etching Agents on Human Dentin. Dent Mater; 1996: 262-271

26. Ramesh Kumar K. R., Shanta Sundari K. K., Venkatesan A., and Shymalaa Chandrasekar. Depth of resin penetration into enamel with 3 types of enamel conditioning methods: A confocal microscopic study. *Am J Orthod Dentofacial Orthop* 2011;140:479-85.
27. Reis AF, Giannini M, Kavaguchi A, Soares CJ, Line SR. Comparison of Microtensile Bond Strength to Enamel and Dentin of Human, Bovine, and Porcine Teeth. *J Adhes Dent* 6; 2004:117-121
28. Rotta M, Bresciani P, Moura SK, Grande RH, Hilgert LA, Baratieri LN, Loguercio AD, Reis A. Effects of Phosphoric Acid Pretreatment and Substitution of Bonding Resin on Bonding Effectiveness of Self-etching Systems to Enamel. *J Adhes Dent* 9; 2007:537-545
29. Silverstone, L. The Acid Etch Technique: In vitro studies with special reference to the Enamel surface and the Enamel-Resin Interface. *Proc. Int. Symp. Acid Etch Tech*;1975:13-39
30. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the Pattern of Acid Etching of Human Dental Enamel Examined by Scanning Electron Microscopy. *Caries Res.* 9;1975:373-387
31. Shade AM, Wajdowicz MN, Bailey CW, Vandewalle KS. The Effect of Simplified Adhesives on the Bond Strength to Dentin of Dual-cure Resin Cements. *Operative Dentistry*; 2014: 627-636

32. Sheykholeslam Z, Buonocore MG. Bonding of Resins to Phosphoric Acid-Etched Enamel Surfaces of Permanent and Deciduous Teeth. J Dent Res;1972:1572-76
33. Sturdevant's Art and Science of Operative Dentistry 4th edition. Pg 181, 238-239, 2002
34. Torres-Gallegos I, Zavala-Alonso V, Patino-Marin N, Martinez-Castanon GN, Anusavice K, Loyola-Rodriguez JP. Enamel roughness and depth profile after phosphoric acid etching of healthy and fluorotic enamel. Australian Dental Journal;2012:151–156.
35. Tyler JE. A scanning Electron Microscope study of factors influencing etch patterns of human enamel. Archs Oral Biol. Vol 21;1976:765-769
36. Wang W, Lu T. Bond strength with various etching times on young permanent teeth. Am J Orthod Dentofacial Orthop; 1991: 72-79
37. Zhu JJ, Tang AH, Matinlinna JP, Hägg U. Acid Etching of Human Enamel in Clinical Applications: A Systemic Review. Journal of Prosthetic Dentistry; 2014:122-135